SPECIFIC ISOTYPE ANTIBODIES OF SECRETION-EXCRETION ANTI-ANTIGENS OF *LEISHMANIA SP* OF PROMASTIGOTE OR AMASTIGOTE FORMS USED AS PROTECTION, RESISTANCE AND CURING MARKERS OF MAMMALS TO LEISHMANIASES AND TO INTRACELLULAR PATHOGENIC MICRO-ORGANISM INFECTIONS, AND AS IMMUNOTHERAPEUTIC EFFECTORS

RELATED U.S. APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

[0001] The present invention involves the detection and the use of IgG2 isotype antibodies specific to secretion-excretion antigens of *Leishmania* which enable:

- proof of a cell-mediated immunity depending on the T lymphocytes of the Th1 type,
- monitoring of the immune response after vaccination or treatment,
- evaluation of the efficacy of a chemotherapeutic and/or immunotherapeutic treatment in mammals and in particular, in humans, canines, felidae, and equidae,
- evaluation of a state of resistance to leishmaniasis,
- establishment of an immunotherapy.

[0002] The present invention also involves :

- proof of a specific humoral response represented by the IgG of isotype IgG_2 in dogs and/or other isotypes in other mammals, a humoral response associated with a cell-mediated response depending on the T lymphocytes of the Th1 type,

- the detection of these IgG specific to the parasite *Leishmania sp* using the well-defined secretion-excretion antigens and

- the neutralizing role of these IgG characterized by the inhibition of the proliferation of the amastigote or promastigote forms of *Leishmania*.

BACKGROUND OF THE INVENTION

[0003] The therapeutic vaccine complex comprised of secretion-excretion products in an axenic and serum-free medium, defined as described in the French patent number 01/07606 dated June 11, 2001, induces an immunostimulation of the lymphocytary system of the Th1 type which ensures the establishment of a protective immunity. This cell-mediated immunity, which is characterized by an activation of the lymphocytes and macrophages and by the synthesis of cytokines specific to the Th1 type, is linked to a humoral immunity characterized by the production of specific IgG which neutralize certain isotypes.

[0004] In a very simple way, it is possible to divide the immune responses into two large qualitatively distinct categories: humoral responses that introduce the production of antibodies by the B lymphocytes, and cellular responses (delayed hypersensitivity reaction, cytotoxic reactions) for which the effective cells are the T lymphocytes. It seems that in the majority of experimental models and clinical situations studied, the cytokines having a lymphocytary origin are essentially produced by the auxiliary T cells CD4+ (or helpers) whose role is to modulate or regulate the humoral and cellular

immunity and which recognize the antigen in association with molecules of class II of the major histocompatibility complex. A major concept emerged in 1985 when T. Mosmann and R. Coffman proposed that the T lymphocytary cells CD4+ expressing the auxiliary functions were in fact heterogens. Thus, via the study of T lymphocytary clones CD4+ of mice, cultivated over a long term, these authors described the existence of two major sub-populations that can be distinguished via their secretion profile from cytokines, i.e. the Th1 cells (for type 1 T helpers) and Th2 cells (for type 2 T helpers).

[0005] Take, for example, leishmaniasis, which is a parasitic infection endemic, or even epidemic, in tropical and subtropical regions of the world. *Leishmania*, flagellate protozoans of the family *Trypanosomatidae* and genus *Leishmania*, are the pathogenic agents responsible for these diseases.

[0006] Numerous studies concerning the immune responses during experimental murine leishmaniases have led to demonstration of the predominant role of cell-mediated immunity and the existence of a duality of the immunological response. There are fundamentally two types of responses against leishmanias: one is described by the sensitivity, the other is described by the resistance. The different subpopulations of T lymphocytes (CD4+) limit or exacerbate the infection by means of the lymphokines they secrete. It has thus been demonstrated that the subpopulation of auxiliary T lymphocytes of the Th1 type (producer of interferon gamma and interleukine 2) was capable of eliminating the amastigote intracellular forms by means of the activation of macrophages (Reiner S.L et al., Annu Rev Immunol, 1995, 13, 151-177. Review). Conversely, the subpopulation of auxiliary T lymphocytes of the type Th2 (producer of interleukine 4) is responsible for exacerbating the disease.

[0007] In humans, certain facts are comparable by nature. In the dog (natural "reservoir" receptive host in the life cycle of *L. infantum*), the duality of the immunological response is likely. Only one study led by Pinelli et al. (Infect. Immun., 62:229, 1994) on animals experimentally and naturally infected by *L. infantum* made it possible to show that the asymptomatism of the dog (clinical state frequently encountered) is accompanied by the absence of a humoral response and the development of a cell-mediated immunity of the Th1 type with a hypersensitivity reaction of the positive delayed type and elevated rates of interleukine 2 and TNF-α circulating in the biological liquids.

[0008] The polarization of the immune responses to a Th1 or Th2 phenotype has been associated with numerous pathological situations.

[0009] For infections by *Leishmania, Trypanozoma, Candida* and other intracellular organisms such as *Mycobacterium* and *Listeria*, a Th1 response correlates to resistance to the pathogen.

BRIEF SUMMARY OF THE INVENTION

[0010] The present invention consists notably in detecting antibodies whose production accompanies the polarization of a cell-mediated immunity to a Th1 type.

[0011] According to specialists like Pinelli (Pinelli. E et al. Infect. Immun, 1994, 62 : 229-235) leishmanian dogs correspond to type Th2 activation of the lymphocytary system having an elevated antibody response. This increased antibody production corresponds to hyperproteinemia and induces the appearance of immune complexes that cause a renal problem (increase in creatinine and blood urea). On the other hand, it has been demonstrated (RIERA, C. et al, vet parasitology, 1999, 84, 1-2, p. 33-47) that the rate of total IgG decreased in experimentally infected dogs after treatment with

glucantime; however, this treatment only induces a temporary recession of symptoms. Relapses remain possible and are not predictable.

[0012] Nevertheless, certain preliminary work in humans (KAWANO. P et al, Parasite Immunol, 1995, 17, 451-458) and in dogs (NIETO C. G. et al, Vet Immunol and Immunopathology, 1999, 67, 117-130) shows that the IgG isotypes would be markers of the Th1/Th2 immunitary dichotomy. In addition, some authors (DEPLAZES. P et al, Parasite Immunology, 1995, 17, p. 451-458 / RIERA, C. et al, vet parasitology, 1999, 84, 1-2, p. 33-47) have studied more specifically the importance of the ratio of isotypes IgG_1/IgG_2 in leishmanian dogs before and after treatment. These IgG_1/IgG_2 were specific to a total somatic antigen of the promastigote, the rate of IgG_2 remained constant before and after treatment; on the other hand, a significant drop of IgG_1 was noticed after treatment.

[0013] However, the specificity of these isotypes to a well-defined antigen or epitope has never been established as the role of these isotypes.

[0014] Dogs that received the therapeutic vaccine complex described in the French patent 01/07606 cited above and thus protected, have significant rates of IgG_2 specific to secretion-excretion proteins, which is compliant with the preferential expansion of T lymphocytes of the Th1 type. On the contrary, the dogs that received a placebo as well as the leishmanian dogs do not have the specific IgG_2.

[0015] The secretion-excretion antigens of the protozoan *Toxoplasma gondii* (patent EP 0 301 961 A) have been studied as vaccines and therapeutic products, but these antigens have never been correlated to the IgG of a particular isotype having a well defined role. On the other hand, some authors (CIBRELUS. P et al, Parasite, 1999, 6 no. 2, p121-129) have shown that naturally infected dogs have total IgG towards the secretion-excretion antigens of *Leishmania infantum* but also no

correlation has been established with immunoglobulins of the specific isotype having a well defined role.

[0016] The present invention also has the purpose of showing that the immunoglobulins of the IgG_2 classes and corresponding sub-classes, linked to the Th1 system, are specific to a well-defined antigen : the secretion-excretion antigens of *Leishmania* (ES), and have a neutralizing action towards the proliferation of amastigotes and promastigotes of *Leishmania sp.*

[0017] These specific IgG_2 induced in dogs immunized with secretion-excretion antigens do not exist in naturally infected mammals. They are thus different from the ones cited in the "Riera" and "Deplazes" documents mentioned above which describe the IgG of naturally leishmanian dogs.

[0018] These IgG_2 are, more precisely, specific to a major protein excreted-secreted by the 2 stages of *Leishmania sp* of the family of the PSA "Protein Surface Antigens" corresponding to a range of molecular mass from 52 to 58 Kda. This major immunogen has an "immunologically silent" epitope during an infection by leishmanias in humans or in dogs. This epitope located in the carboxyterminal preserved region is recognized by the IgG in dogs. Some authors (KEMP. M et al, FEMS Immunol and med microbiology, Netherlands, 1998, 20 no. 3, p 209-218) have demonstrated the existence of a glycoprotein of surface PSA-2 in *Leishmania major* responsible for cutaneous leishmaniasis in humans and in mice. This glycoprotein that corresponds to a constituent surface antigen of the promastigote form of the parasite and not of excretion-secretion antigens, induces an immunitary state of the Th1 type in mice. Other teams (JIMENEZ-RUIZ. A et al, Europ J of Biochemistry / FEBS Germany, 1998, 25 no. 1-2, p 389-397) demonstrate that a PSA of a promastigote of *Leishmania infantum* is a major immunogen of the B cells in naturally infected dogs. In the two cases, the specificity of IgG_2 having a neutralizing activity has not been proven. Moreover, the antibody

response during a natural infection according to JIMENEZ-RUIZ. A et al. is essentially directed against the repeated patterns rich in Leucine, and not against the carboxyterminal part, the major antigen of the surface of *Leishmania infantum* (and thus not of an excretion-secretion antigen).

[0019] The major antigen of the excretion-secretion products has been characterized by the elaboration of monoclonal antibodies (AMC), and it belongs to the family of the PSA and corresponds to the range of molecular mass from 52 to 58 Kda. Two banks of ADNc expression of the promastigote forms and amastigote forms of Leishmania have been immunologically separated using AMC F5 in order to identify the major immunogens of the AES. This has made it possible to isolate ADNc from a given clone which codes an excreted-secreted protein of the PSA family.

[0020] These PSA proteins have characteristic repeated domains rich in leucine.

[0021] The analysis of ADNc sequences reveals a peptide sequence corresponding exactly to the COOH-terminal end of the isolated PSAs (see Figure 1 : schematic representation of the COOH terminal end of PSAs identified by the monoclonal antibody F5).

[0022] Using a recombinant protein coding for the carboxyterminal part of the PSAs, it has been shown that patients or dogs which have contracted leishmaniasis are incapable of producing antibodies against the epitopes carried by the recombinant protein (see Figure 2 : Analysis of the humoral response of patients and dogs naturally infected with regard to the recombinant protein 6 (His)-COOH-PSA), while they produce antibodies against native ES antigens, thus against other epitopes present on the native PSAs. On the contrary, dogs immunized with AESs of promastigotes of *Leishmania infantum*, and protected against visceral leishmaniasis, have antibodies of the isotype IgG2 specific to the carboxyterminal part (see Figure 3 : Analysis of the humoral response of dogs

immunized by the AESs of promastigotes of *Leishmania infantum* relative to the recombinant protein 6 (His)-COOH-PSA).

[0023] The present invention also has the purpose of showing that after chemotherapy or immunotherapy, the IgG2s in dogs specific to the ES antigens of Leishmania and notably of the carboxyterminal part of PSA appear with significant improvement in the general state of dogs that have contracted *Leishmaniasis*.

DETAILED DESCRIPTION OF THE INVENTION

[0024] As for all vector-transmitted diseases, leishmaniases are characterized by a life cycle that is relatively simple since it is divided between two hosts, mammals and insects (phlebotomes), and consists of two main forms:

- a flagellate form called a promastigote, present in the digestive tract of the phlebotomic vector, where it multiplies prior to acquiring its form that is infectious for the mammalian host, also called the metacyclic form;
- a non-flagellate form called amastigote, present in the mammalian host such as the dog and human.

[0025] In order to study the synthesis specific to the antibody IgG_2 in dogs, 2 types of experiments were performed after immunization by the therapeutic vaccine complex comprised of ES : role of the *in vitro* effect of the immune serum of dogs on the kinetics of multiplication of the different stages of the parasite *Leishmania infantum* ; dosage of the IgG_2 specific excretion-secretion anti-antigens of *Leishmania*.

[0026] For our experiments, we used the vaccine complex composed of excretion-secretion antigens of promastigotes noted in the French patent application no. 01/07606 mentioned above.

[0027] The dogs are immunized according to the following scheme :

4 weeks	4 weeks	
1st injection ----------> sub-cutaneous	2nd injection----------> sub-cutaneous	blood sampling to study the immune serums and antibodies.

[0028] Another type of experiment was performed : dosage of the specific IgG2 anti antigens ES of *Leishmania* in leishmanian dogs treated by chemotherapy. For this experiment, we used, as the antigen, the recombinant protein coding for the carboxyterminal part of PSA.

[0029] The methods used are the following :

[0030] a. Parasitic material :

[0031] The culture of promastigotes of *leishmania infantum* MON. 1 is collected in a stationary phase of growth. The parasites are washed 3 times in 15 ml of sterile PBS (centrifugation at 4 °C for 5000 revolutions over 10 min) then collected in 1 ml of PBS.

[0032] The promastigote/amastigote differentiation and thus the multiplication of the amastigotes requires 0.5 x 10^6 parasites per ml of culture medium MAA20 pH 5.8 in a culture chamber incubated at 37 °C and under 5% CO2 (conditions representing the phagolysosomial environment) ; the proliferation of promastigotes requires 10^6 parasites per ml of culture medium RPMI 20% pH 7.2 in a culture chamber incubated at 25 °C (conditions representing the phlebotome).

[0033] A viability test with Trypan® Blue at 0.4% in PBS and a Thomas cell counting must be done in order to resuspend the parasites in sterile PBS so that they are at 10^6/ml.

[0034] b. Preparation of the serums :

[0035] One aliquot of each serum is decomplemented by passage through a water bath for 45 min. at 56 °C then centrifuged 12 min for 12000 revolutions in order to prevent agglutinins.

[0036] First, with the serumal ratio in antibody not being known, a range of aleatory dilution is performed. For 10^6 parasites, it is necessary to put it in contact with 20 µl of serum:

- 1st test pure serum: 5.10^6 parasites (divided into RPMI and MAA) i.e. $(5.10^6 \times 20)/10^6 = 100$ µl of pure serum.
- 2nd test dilution to ½: 50 µl of pure serum + 50 µl of sterile PBS buffer.
- 3rd test dilution to ¼: 25 µl of pure serum + 75 µl of sterile PBS.
- 4th test dilution to 1/8 : 12.5 µl of pure serum + 87.5 µl of sterile PBS

[0037] c. Contact Promastigotes-Antibody:

[0038] The contacts are made in 1.5 ml Eppendorff tubes where 5.10^6 parasites are treated with 100 µl of serum for 30 min. over ice (minimum time necessary for a promastigote to infect a macrophage). The excess antibody is then washed twice with 1 ml of PBS and by a centrifugation for 5000 revolutions for 10 min., repeated twice.

[0039] d. Analyses :

[0040] One aliquot of 10 ul for each test is then taken immediately after contact in order to perform a viability test with Trypan® Blue and a Thomas cell counting, the surplus serving for the cultivation from which a counting is done daily.

[0041]

- **Proliferation of the promastigotes** (medium: RPMI 20%)

[0042] A Thomas cell counting from each test is done daily in order to monitor the kinetics of multiplication.

[0043] From an aliquot of each cultivation test, a parasitic smear fixed with methanol then colored with May-Grunvald-Giemsa is done daily, whereby the percentage of the promastigote form,

spheromastigote form and amastigote form is determined by counting 100 parasites.

[0044]

- **Proliferation of the amastigotes** (medium: MAA 20)

[0045] From a second aliquot of each cultivation test, a Thomas cell counting is done in order to monitor the kinetics of multiplication.

[0046] The calculations of inhibition of growth are done on the following model:

The number of parasites treated with the healthy serum (= Ns) → 100% of growth

The number of parasites treated with the immunized serum (=Ni) → x% of growth

% inhibition = 100 - (Ni x100)/Ns

[0047] e. Studies of the antibodies :

[0048] The total IgG antibodies in dogs antiLeishmania are detected by traditional immunofluorescence using slides coated with promastigotes (serological reference method for canine leishmaniasis).

[0049] The detection of the IgG2 specific to ESP is done by ELISA according to the technique of microtitration by Kweider et al (J. immunol, 1987, 138 : 299) using the ESP as antigens and a conjugated antiIgG2.

[0050] f. Analyses performed in dogs immunized with the ESP :

[0051] A complete inhibition study of the proliferation of amastigotes and promastigotes was done on two dogs :

The dog named MINON : Weimaraner breed, female, 4 years old, vaccinated with ESP

The dog named MUMA : Brittany Spaniel breed, female, 7 years old, control placebo

[0052] A viability test of the promastigotes was done on 6 other dogs in an experimental study and

4 dogs in a local environment study (test of the vaccine complex ES in the endemic zone) compared to 2 control dogs.

[0053] g. Study of antibodies in leishmanian dogs treated by chemotherapy :

[0054] For the 3rd experiment, serums of 5 dogs infected with visceral leishmaniasis and treated by glucantime for 1 to 2 months, coming from Dr B, were analyzed.

[0055] h. Studies of antibodies in leishmanian dogs treated by immunotherapy with ESP :

[0056] Three leishmanian dogs were treated by subcutaneous injection of ESP (3 injections of 50 µg, each injection two weeks apart).

[0057] The total antibodies IgG in dogs anti Leishmania were detected by immunofluorescence and the detection of antibodies IgG2 specific to the ESPs was done by ELISA as described above (see : e.: Study of antibodies) but using as an antigen the recombinant protein coding for the carboxyterminal part of the PSA.

[0058] RESULTS :

[0059] *1. Study of the proliferation of promastigotes of L.infantum :*

[0060] • The following study was done on parasites put into contact with a serum of a healthy dog or a dog immunized 4 times with the excretion-secretion products of promastigotes (ESP) and before challenge.

[0061] Determined by a test with Trypan® Blue, the viability of the promastigotes was 100% before contact with the serum. After 30 min. of contact, the viability with the healthy serum was always 100% at all dilutions, while with the serum immunized with ESP, it was only more than 50% with the pure serum, 73% with the serum diluted to ½, 92% and 94% with respectively the serum diluted to ¼ and 1/8 (see Figure 4 : Kinetics of proliferation of promastigotes after 30 minutes of contact with

different dilutions of serums (Pure (A); ½ (B) ; ¼ (C) ; 1/8 (D)) in the healthy dog (S) Muma and immunized with the ESP(I) Minon).

[0062] The following table describes the development of the percentage of inhibition of the growth of promastigotes after contact of 30 minutes with serum of the immunized dog (ESP) relative to the serum of the healthy dog.

Percentage	**1st day**	**2nd day**	**3rd day**	**4th day**	**Averagel**
inhibition					
pure / S pure	93%	92%	98%	73%	89%
I ½ / S ½	94%	94%	84%	90%	90%
I ¼ / S ¼	79%	60%	45%	0%	46%
I 1/8 / S 1/8	ND	81%	90%	81%	84%

[0063] • **Interpretation :**

[0064] The inhibition of growth with the anti-ESP serum (the dog MINON) relative to the healthy serum approaches 90% regardless of the dose (aside from for the dilution ¼, manipulation errors ?). It appears that the threshold value of activity of this serum is not obtained with this range of dilutions, at least the cytotoxic effect is powerful and the growth of promastigotes is greatly slowed down for all dilutions of the serums.

[0065] *2. Study on the proliferation of amastigotes of L. infantum*

[0066] • The following study was done on cultures in MAA. 20 for differentiation into amastigotes, from promastigotes treated with a serum before challenge of a healthy dog or a dog immunized 4 times with the ESP (see Figure 5 : Kinetics of proliferation of amastigotes after 30 minutes of contact with different dilutions of serums (Pure (A); ½ (B) ; ¼ (C) ; 1/8 (D)) of the healthy dog (S) Muma and the dog immunized with ESP(I) Minon).

[0067] The following table indicates the percentages of inhibition of the multiplication of the amastigotes after 30 minutes of contact with the serum of the immunized dog (ESP), relative to the serum of the healthy dog :

% inhibition	**1st day**	**2nd day**	**3rd day**	**Average**
Ip/Sp	66%	68%	61%	65%
I ½ / S ½	47%	60%	42%	51%
I ¼ / S ¼	24%	47%	47%	39%
I 1/8 / S 1/8	15%	18%	24%	19%

[0068] • **Interpretations :**

[0069] An inhibition of the growth is noticed on the kinetics of growth of amastigotes when before differentiation, the promastigotes have been put in contact with an anti-ESP serum. The growth of amastigotes is then slowed down and this in a manner proportional to the concentration of serum. The more the serum of the dog immunized to ESP is concentrated, the more the multiplication of the amastigotes is inhibited. Thus, there is a dosage-dependence effect.

[0070] *3. Results obtained in other dogs : dogs immunized with ESP and protected against experimental infection and natural infection.*

[0071] The average viability of the promastigotes after contact with the serum of the dog immunized vaccine complex ESP is 16.8% (5 dogs vaccinated) and 72.9% (5 dogs placebo) for serum of non-immunized dogs. Also, antibodies of the immunized dog not linked to the complement are cytotoxic for the promastigotes.

[0072] The following table indicates the percentages of viability of promastigotes after contact, with the serums of immunized dogs and non-immunized dogs with ESP.

Dogs analyzed	T1* (control)	T2* (control)	**Experimental Studies (experimental infection)**					
			E	O	G	K	7	16
			Placebo	Placebo	Vaccine	Vaccine	Vaccine	Placebo
Percentage viability	100%	100%	85.7%	61.9%	22.4%	10.1%	12.8%	76.2%

Dogs analyzed	T1* (control)	T2* (control)	**Studies in local environment (natural infection)**			
			HC 046	HC 051	LB 120	RR 019
			Placebo	Vaccine	Placebo	Vaccine
Percentage viability	100%	100%	66.7%	15.7%	73.8%	22.9%

[0073] • Dogs T1 and T2 : healthy non-immunized dogs which did not receive the placebo.

[0074] *4. Correlation between production of IgG_2 anti ESP and the effect of the serums on the viability of the promastigotes*

[0075] According to the table below (Correlation IgG2 anti ESP/inhibition proliferation of the promastigotes), the fall in the percentage of viability of the promastigotes corresponds to the presence of IgG2 specific to the vaccine complex ESP.

Dogs experimentally infected	MINON	MUMA	E	O	G	K	7	16
IgG total By IF*	-	-	-	-	-	-	-	-
IgG2 specific to ESP** by ELISA	**0.391**	0.108	0.065	0.104	**0.299**	**0.299**	**0.310**	0.056
Percentage viability	50%	100%	85.7%	61.9%	22.4%	10.1%	12.8%	76.2%

Dogs naturally infected	**HC 046**	**HC 051**	**LB 120**	**RR 019**
IgG total By IF*	-	-	-	-
IgG2 specific to ESP** by ELISA	0.054	**0.321**	0.065	**0.386**
Percentage viability	66.7%	15.7%	73.8%	22.9%

* IF : Immunofluorescence : negative : rate ≤ 1/100

** IgG_2 : DO at 492 nm : the numbers in boldface correspond to positive results

[0076] *5. Study of the IgG2 in leishmanian dogs treated by chemotherapy :*

[0077] The 5 leishmanian dogs had, at the start of treatment, a higher rate of total IgG (≥ 1/200), this rate stayed fixed or dropped to 1/50 4 months after the start of treatment for 4 dogs.

[0078] The fifth one died 2 months after treatment, he had an increase in total IgG (1/200 to 1/800) and was negative in IgG_2 directed against the carboxyterminal part of PSA.

[0079] On the contrary, the 4 other dogs recovered and have IgG_2 against the carboxyterminal part of the PSA.

[0080] *6. Study of IgG_2 in leishmanian dogs treated by immunotherapy with ESP :*

[0081] The three dogs have at the start of treatment a very high rate of total IgG. This rate decreases 3 months after treatment. In parallel to this drop in total IgG, the clinical signs become unclear and IgG2s specific to the product injected appear (see table below) :

Leishmanian dogs		LAFAYETTE	JERRY	RODEO
IgG total by IF*	Before treatment	1/800	1/3200	1/800
	After treatment	1/200	1/400	1/100
IgG specific to ESP by ELISA**	Before treatment	0.052	0.060	0.059
	After treatment	0.361	0.327	0.359

* IF : Immunofluorescence : negative rate ≤ 1/100

** IgG2 : DO at 492 nm, the underlined characters correspond to positive results.

[0082] It thus appears that the capacity to produce the IgG_2 antibodies against this epitope specific to PSA accompanies the evolution towards recovery of the dogs. Thus, it is indeed the IgG_2s which are responsible for the lysis of the amastigotes and promastigotes of *Leishmania sp* in vitro and for the neutralization of their proliferation.

[0083] The analysis of the serums of 3 leishmanian dogs treated by the ESP vaccine complex gives exactly the same type of result, i.e. an appearance of IgG_2s specific (to the carboxyterminal part of PSA of the ESP) correlating with an activity of the serum neutralizing the proliferation of the promastigotes of *Leishmania infantum* in vitro and a cell-mediated immunity of Th1 type.

[0084] These IgG_2, in dogs, or other particular isotypes in the other mammals associated with a cellular immunity of the Th1 type, can be detected by various in vitro methods, for example : ELISA, DOT BLOT, WESTERN BLOT, IMMUNOCHROMATOGRAPHY, IMMUNO NEPHELOMETRY, RIA, IMMUNO PRECIPITATION, ELECTRO SYNERESIS and any other in vitro method that causes intervention of a conjugated system or other systems of visualization for the Ag-Ac reaction.

Thus, the immunoglobulins according to the invention can be used for a diagnostic product that makes it possible to detect one or more epitopes carried by the terminal ends NH_2 and COOH of the Protein Surface Antigen excreted-secreted by *Leishmania sp.* For example, an ELISA system can be used on a plastic support and a WESTERN BLOT system on membranes of nitrocellulose or other polymers that cause an enzymatic conjugate to intervene. Latex supports can also be used. Some radio-isotopes, fluorescent molecules, luminescent molecules or particles of color can be coupled to these various antigens to be used as conjugates. Some metallic colloids can also be used. In addition, excretion-secretion antigens of amastigotes or promastigotes of *Leishmania sp* and more particularly the carboxyterminal part of the Protein Surface Antigen in purified form or recombinant protein form are coupled to the large molecules of biotin, avidin, streptavidine or any other protein to make them more accessible.

[0085] Thus, antibodies of the isotype IgG2 and the corresponding sub-classes according to the invention can be used in mammals as markers of an immunitary state of the cell-mediated type depending on T lymphocytes and preferably the T lymphocytes of the Th1 type, like markers of the resistance to leishmaniasis and to infections by pathogenic intracellular micro-organisms in mammals, as markers of the immunoprophylactic and immunotherapeutic vaccination for infections by pathogenic intracellular micro-organisms, and as effectors of immunotherapy for leishmaniases and infections by pathogenic intracellular micro-organisms.